Instructions: *Use Times New Roman font size 11, 1.0 pt text space, and maximum three A4 pages, including any figures.*

Name or type of instrument	**Orbitrap Ultrahigh Resolution Mass Spectrometer (Orbitrap uHRMS)**
Unit	Exposomics
Platform	Metabolomics
Applicant (Head of Unit)	Dr. Stefano Papazian (HoU), and Prof. Jonathan W Martin (Scientific Director)
E-mail address	stefano.papazian@scilifelab.se; jon.martin@scilifelab.se
Instrument, acquisition cost (kSEK)	6014
Funding applied for (kSEK)	3007 (50%)

Description of the Instrument

**NB, this application from the Metabolomics Platform is ranked #1 internal priority, and the Platform has not previously received funding from this Expensive Instruments program.*

Instrument Description and Purpose. The requested instrument is an Orbitrap ultrahigh resolution mass spectrometer (Orbitrap uHRMS). The Orbitrap uHRMS will be interfaced to an ultra high-pressure liquid chromatograph (LC) through an atmospheric pressure inlet, including electrospray (ESI) and atmospheric pressure chemical ionization (APCI). This instrumentation will be dedicated for comprehensive small molecule detection and characterization in complex human and environmental samples, such as extracts of human plasma, urine, air samples, or environmental waters. The requested instrument is both: (i) highly sensitive for targeted detection of trace environmental pollutants and endogenous metabolites, and (ii) the best available technology in terms of resolution for untargeted identification of small molecules in complex mixtures.

A Unique and Essential Instrument for National Service. The Metabolomics Platform has no other uHRMS instruments. The requested instrument will be the work-horse instrument for the Exposomics Unit and must be purchased to offer our basic scientific service. Last year this application was not reviewed, because we were deemed ineligible as a pilot unit, but now we have submitted our interim report and already demonstrated a significant user base in Sweden and internationally. This early progress was accomplished by borrowing Orbitrap time from the SU exposomics research group (Prof. Martin), but this strategy has delayed thesis research projects and is not sustainable. To solve instrument overbooking, Prof. Martin has proceeded with procurement of the current uHRMS instrument, which was recently installed (Aug-22, first invoice expected Oct-22). The agreement is that half the instrument time will be devoted to the Exposomics Unit, hence the 50% funding request.

An LC-Orbitrap uHRMS hosted at the Exposomics Unit will be the first of its kind at the Metabolomics Platform. The Global Proteomics and Proteogenomics Platform (Campus Solna) already has several uHRMS Orbitrap instruments, including the exact model requested here, but their nano-LC setups are not compatible with our analytical flows. In short, this state-of-the-art instrument will be unique to national users of the Exposomics Unit and will support cross-unit initiatives at the Platform, including the recent Exposome-Metabolome Interaction grants.

Instrument Details. The specific uHRMS requested is a new generation **Orbitrap Exploris 480**, which has mass resolution (m/Δm) of up to 480,000 at mass to charge (*m/z*) 200. Orbitrap mass

analyzers are a commercial technology available from Thermo Scientific, and compared to quadrupole time-of-flight (QTOF) detectors, which reach highest resolving powers for large molecules (e.g. proteins) Orbitrap achieves ultrahigh resolution (i.e. m/Δm >100,000) for small molecules which are the target molecules of exposomics and metabolomics workflows.[1]

The Critical Importance of Resolution for Accurate Molecular Annotation. Competing modern HRMS QTOF instruments (including at the Swedish Metabolomics Centre) reach a maximum mass resolution in the range of 40,000-70,000, which is not high enough to adequately resolve complex small molecule mixtures. This is unacceptable for exposomics in complex samples, such as biofluids,[2] where major endogenous metabolites can easily mask co-eluting small molecule environmental contaminants at 1000× lower concentrations, but with similar *m/z*. Adequate resolution is also critical for correct molecular formula assignments (e.g. $C_xH_yO_zN_aS_b$) to thousands of mass spectral features with unknown identity. One of the most commonly encountered challenges in molecular formula assignment for biological and environmental samples is differentiating the 3.4 mDa mass split between three carbon atoms (i.e. C_3, 36.0000 u) and a sulfur with 4 hydrogen (SH_4 36.003372 u)[3]; this task cannot be overcome with QTOF, which thus creates ambiguity for correctly annotating sulfur containing metabolites, such as sulfates and thiols. Orbitrap mass spectrometers operating above 120,000 resolution can easily differentiate and correctly annotate such molecules.[3] At even higher resolutions (i.e. m/Δm >400,000) of the requested instrument, Orbitrap can more effectively annotate molecules by their fine isotopic patterns.[4]

With the Orbitrap Exploris 480, correct formula assignments are moreover aided by sub part-per-million (ppm) mass accuracy (i.e. <1 ppm error in m/z) using a flow of internal calibrant ions, which allows real-time *m/z* correction throughout data acquisition. The Exploris 480 can also acquire in a range of dynamic modes in parallel, at up to 40 Hz, including full scan, MS/MS (data-dependent, or data-independent), as well as in targeted modes. A typical Orbitrap workflow on the Exploris 480 will allow high-resolution (240-480k) full scan MS^1 acquisition in parallel with quadrupole precursor isolation and MS^2 spectral fragmentation in the collision cell, allowing structural elucidation of most unknowns. Together, these features make the Exploris 480 a very dynamic and suitable instrument for our combined target and nontarget workflows now being delivered in the Exposomics Unit.

A Dynamic and Highly Sensitive Instrument. The Orbitrap Exploris 480 is the most sensitive instrument in the model line-up (i.e. compared to Exploris 120 and 240 models) because of its high-capacity ion transfer tube, which maximizes ion flux from the ionization source into the high vacuum regions of the ion optics. Sensitivity specifications are listed as 50 femtograms reserpine on column, with signal to noise ratio greater than 100:1. This impressive instrumental sensitivity is of particular importance in exposomics for detection of anthropogenic chemical exposures, which are typically in the range of 100-1000x lower than endogenous metabolites.[2] The added benefit of this sensitivity is that we can also detect important trace metabolites (e.g. sex steroids and thyroid hormones) which are known to be disrupted in background human populations by exposure to common endocrine disruptors[5] such as brominated flame retardants, bisphenol A, and per- and poly- fluoroalkyl substances (PFAS). New such studies of exposome-metabolome interaction, enabled by our Unit, are creating new synergies within the Metabolomics Platform that will be of high interest to Swedish researchers and the international research community.

Statement of Need

A Crucial Instrument for Exposomics, and New Technology at the Platform. The Metabolomics Platform does not currently have any uHRMS instruments, and the new Exposomics pilot facility cannot operate sustainably without dedicated access to at least one instrument. The requested instrument will be the ‘work-horse’ of our LC-based chemical exposome services, but will also be available on a priority basis to other Metabolomics Platform users through the Swedish Metabolomics Centre (Umeå) and Chalmers Mass Spectrometry Infrastructure (Gothenburg). The current proposal for instrumentation is complemented by the Metabolomics Platform TDP grant (led by Hans Stenlund,

funded 2021), which is now building common open science pipelines to allow FAIR data sharing and processing for our staff and users. Overall, this instrument will enable technological development and support new studies of the exposome-metabolome interaction at the national level.

Synergy with SciLifeLab Research. The Martin exposomics research group is currently composed of 8 members who depend highly on another HPLC-Orbitrap (Q-Exactive HFX, 2017) for workflow development and applied exposome studies. National users of the Exposomics Unit are already providing hundreds of samples that need to be analysed in sequence for months at a time, thus necessitating a dedicated HPLC-Orbitrap instrument for national service. The expanding Exposomics Unit will benefit from synergy with its neighbouring research program (and *vice versa*), thus increasing the efficiency and quality of the national service. For instance, technologies that have been developed in the research group can be easily translated to the Orbitrap Exploris 480 for unique national services, not yet available elsewhere in the world. Postdoctoral fellows and PhD students have already optimized protocols for the analysis of blood plasma and water, which are now being provided as service to our users. Moreover, the experience of Martin´s research group in data processing with open science software will be shared with users and staff of the Metabolomics Platform through collaborative workshops.

Summary of Expected Impacts From A User Perspective:

- Dedicated instrument will allow efficient sample analysis and data reporting, and users should be happy to return with future projects.
- Best available uHRMS data quality (resolution, sensitivity) will enable higher rates of molecular annotation in studies of national importance, thereby increasing discoveries and raising impact of Swedish scientific work.
- New services of national interest will become available, including studies of exposome-metabolome interaction (within platform), and exposome-genome interaction (across SciLifeLab platforms).

Physical Placement of the Instrument: The current instrument has already arrived and is in place in the newly renovated MS-Lab on Alpha-1.

Base Funding and Financial Plan of Exposomics Unit: Base budget for Exposomics was 1.9 MSEK (2021) and 1.8 MSEK (2022), and a rapid increase in our Unit staff was recommended by the IAB evaluation in 2021. The base-budget primarily covers full-time salary and social benefit costs for the Head of Unit (Stefano Papazian), with the balance being consumed if we must put it towards depreciation costs of the new instrument. In the current proposal we have requested 50% of the purchase cost, which will give us the needed flexibility to commit to an additional full-time laboratory manager to focus on laboratory work and instrumental maintenance, and thereby giving the HoU a much-needed full-time colleague to help deliver our services.

Co-Funding from SU (4763 kSEK): Prof Martin will pay 50% (3007 kSEK) of costs from his research funding. Stockholm University has committed 2000 kSEK to support the launch and operations of the Exposomics Unit. Most of this co-funding will pay the service contract (1756 kSEK) on the requested instrument, with the balance needed to invest in multiwell equipment at the unit.

Literature Cited:

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